PRODUCTION OF BIOGAS FROM POULTRY MANURE WASTEWATER: OPTIMIZATION

CHOO WEI CHUN

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JANUARY 2015

ABSTRACT

An exaggeration increment of population has led to depletion of energy resources. One of the most effective solutions is to implement the uses of renewable energy sources. In particular, biomass, which can be further converted into biofuel, is normally derived from plants. However, it brings several drawbacks which may directly threaten the sensitive species. To alleviate these, biogas production from poultry manure wastewater was explored in the current work. Its usage to produce biogas was considered as a triumph to the concept of waste-to-wealth. The main objective of this research study was the optimization of biogas production from poultry manure wastewater by soil mixed culture. The poultry manure collected was gone through characterization and pre-treatment processes to remove excessive ammonia-N which cause inhibition to the biogas production. The optimization was analyzed by central composite design (CCD). Previous studies have screened out five processing parameters, which were agitation speed, reaction time, substrate to inoculum ratio, process system and type of substrate. Significantly, it had been identified that agitation speed and reaction time were the most crucial parameters. The best screening condition obtained from previous studies was 120 rpm agitation speed and 3 days of reaction time. Consequently, there were two factors involved in current research study, which are agitation speeds ranged from 100 rpm to 140 rpm; and reaction time ranged from 2 days to 5 days. The biogas production was collected by water displacement experimental set up. In addition, chemical oxygen demand (COD) value was determined using HACH DR5000 spectrophotometer with the aid of COD digestion reactor. Lastly, the experiment was designed and analyzed by Design Expert V7.0 software using response surface methodology (RSM). The biogas production performance was evaluated on the basis of biogas yield from initial COD and was found ranging from 0.49 to 4.37 mL/g COD. Quadratic model was well fitted (R-squared>0.80) with a confidence level higher than 95 %. For validation run, the optimum biogas production was using agitation: 120 rpm and reaction time: 3.3 days. Under this condition, 4.45 mL/g COD of biogas yield was obtained. This counted for 5.82 % error from predicted models. It is recommended to construct a pilot study of scale-up experiment for the optimization of biogas production under optimum conditions obtained from this study.

ABSTRAK

Populasi penduduk yang semakin meningkat telah menyebabkan penyusutan sumber tenaga. Salah satu cara penyelesaian yang paling berkesan adalah dengan melaksanakan penggunaan sumber tenaga boleh diperbaharui. Khususnya, biojisim, yang boleh terus ditukar menjadi bahan api bio, biasanya diperolehi daripada tumbuhan. Walau bagaimanapun, ia membawa beberapa kelemahan yang secara langsung boleh mengancam beberapa spesies tumbuhan. Untuk mengatasi masalah ini, penghasilan biogas daripada najis ayam akan diterokai dalam kerja kajian ini. Penggunaannya untuk menghasilkan biogas akan dianggap sebagai kemenangan kepada konsep sisa kepada kekayaan. Objektif utama kajian penyelidikan ini adalah pengoptimuman penghasilan biogas daripada najis ayam oleh kultur tanah campuran. Najis ayam yang diperolehi telah melalui proses pencirian dan pra-rawatan untuk mengurangkan ammonia-N yang berlebihan yang akan menyebabkan perencatan untuk pengeluaran biogas. Pengoptimuman telah dianalisis oleh Reka Bentuk Komposit Pusat (CCD). Kajian sebelum ini telah disaringkan dengan lima parameter pemprosesan, iaitu kelajuan pergolakan, masa tindak balas, nisbah substrat kepada inokulum, sistem proses dan jenis substrat. Dengan ketara, ia telah dikenal pasti bahawa kelajuan pergolakan dan masa tindak balas merupakan parameter yang paling penting. Keadaan penyaringan terbaik yang diperolehi daripada kajian sebelum ini ialah kelajuan pergolakan dengan 120 rpm dan masa tindak balas dengan 3 hari. Oleh yang demikian, terdapat dua faktor yang terlibat dalam kajian penyelidikan semasa, iaitu kelajuan pergolakan antara 100 rpm hingga 140 rpm; dan masa tindak balas antara 2 hari hingga 5 hari. Penghasilan biogas telah dikumpulkan dengan teknik eksperimen anjakan air. Selain itu, nilai permintaan oksigen kimia (COD) telah ditentukan dengan menggunakan spektrofotometer HACH DR5000 dengan bantuan COD penghadaman reaktor. Akhir sekali, eksperimen telah direka dan dianalisis oleh perisian Design Expert versi 7.0 menggunakan metodologi permukaan tindak balas (RSM). Prestasi penghasilan biogas telah dinilai atas dasar hasil biogas daripada COD awal dan didapati dari 0.49 untuk 4.37 mL/g COD. Model kuadratik telah dipasang dengan baik (R-kuasa dua> 0.80) dengan tahap keyakinan yang lebih tinggi daripada 95%. Untuk eksperimen pengesahan, penghasilan biogas optimum adalah menggunakan pergolakan: 120 rpm dan tindak balas masa: 3.3 hari. Di bawah keadaan ini, 4.45 mL/g COD hasil biogas diperolehi. Ini diambil kira untuk kesilapan 5.82% daripada model yang diramalkan. Adalah disyorkan untuk membina satu kajian perintis eksperimen meningkatkan skala untuk mengoptimumkan pengeluaran biogas di bawah keadaan optimum yang diperolehi dari kajian ini .

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD	Anaerobic digestion
AN	Ammoniacal Nitrogen
ANOVA	Analysis of variance
ASBR	Anaerobic sequencing batch reactor
BOD	Biochemical Oxygen Demand
CCD	Central Composite Design
CHP	Combined heat and power
CM	Chicken manure
COD	Chemical Oxygen Demand
DF	Degree of freedom
FFD	Full Factorial Design
F/M	Food-to-microorganism ratio
GHG	Greenhouse gases
HRT	Hydraulic retention time
LSD	Least Significant Difference
MSW	Municipal solid waste
NPK	Nitrogen: Phosphorus: Potassium ratio
OHPA	Obligatory Hydrogen-Producing acetogens
OLR	Organic Loading Rate
PMW	Poultry Manure Wastewater
PS	Peat soil
RSM	Response Surface Methodology
S/I	Substrate to inoculum ratio
SMC	Soil mixed culture
SS	Suspended solid
SW	Soil water

Greek

k	parameter number
c_p	replicate number of central point
α	coded factor
R^2	coefficient of determination
e_i	residuals
y_i	difference between the actual individual values

1 INTRODUCTION

1.1 Motivation and statement of problem

Biogas, a gas mixture produced by the decomposition of organic matter in anaerobic condition, is introduced to act as an alternative renewable energy source (Ahn *et al*, 2010). It contains on an average distribution of 50-70% methane, 30-40% carbon dioxide, 1-2% nitrogen, 5-10% hydrogen, trace amounts of hydrogen sulfide and water vapor (Grant and Marshalleck, 2008).

The annual production of poultry and livestock manure on centralized farms (216,000 pigs per year, 15,000 beef per years and up to 4 million chickens per year) in Russia overreached 700 million m^3 and led to severe environmental issues such as foul odor problems due to high levels of ammonia, attraction of rodents and pathogenic microorganisms, runoff of phosphorus into water source and groundwater contamination due to nitrate leaching (Kalyuzhnyi *et al.*, 1998; Atuanya and Aigbirior, 2002). According to the Polish Act of 10 July 2007 on fertilizers and fertilizing, the Construction Law (Journal of Laws of 2006, No. 156, item 1118), poultry farmers are obliged to dispose a minimum of 70% of poultry manure on farms and store the manure in sealed containers with capacity of at least four months of fertilizer production (Borowski *et al*., 2014).The utilization of poultry manure wastewater to produce biogas via anaerobic digestion can help to resolve the overwhelmed manure in farms (Sakar *et al.,* 2009).

The motivation of utilization of poultry manure for anaerobic digestion (AD) instead of other manures to produce biogas is due to its high nutrient contents (Roeper *et al.,* 2005). According to FFTC Annual Report 2011 (2012), the approximate ratio value of Nitrogen: Phosphorus: Potassium (NPK) on dry basis matter of dairy cow dung is 2.19: 1.37: 0.67%; for swine is 2.91: 2.85: 1.38%; and highest for poultry which is 4.34: 4.41: 2.24%, respectively. Although poultry manure can be characterize as one of the best organic fertilizer sources, excessive implementation may lead to severe environmental issues (Kalyuzhnyi *et al.,* 1998).

In addition, the AD of biomass requires lower capital and operating cost as compared to other renewable energy sources (Rao *et al.,* 2010). This technology having great potential in pathogen levels reduction, odor regulation and fertilizer value improvement to alleviate both economic and environmental issues (Demirer and Chen, 2005). The end products of AD are biogas and digestate. The methane concentrations contain in biogas yield from AD of manure or biodegradable waste can be up to 80% in volume whereas the digestate is a moist solid bringing fertilizer values (Demirbas *et al.,* 2011). As a bio-renewable energy source, biogas produced can be used to generate heat and electricity (Angelidaki and Ahring, 1997).

In order to optimize this model with a number of interrelated parameters in one time, RSM conducted by process modeling and optimization has been introduced (Rastegar *et al.,* 2011). RSM is an optimization method which collects a group of mathematical and statistical techniques to define the relationships between the response and the independent variables. It is divided into three major stages which are preliminary determination of independent parameters and levels, selection of experimental design, and graphical presentation of result analysis (Ba ş and Boyacı, 2007). Lastly, the significance of the design model will be analysed by the coefficient of determination (R^2) from the analysis of variance (ANOVA) which determine the quality of the fit of quadratic correlations (Amani *et al.,* 2012).

1.2 Objectives

The main objective of this study is to optimize the production of biogas from poultry manure wastewater.

1.3 Scope of this research

Chicken manure (CM) wastewater sample will be collected from local poultry manure farm, before the pre-treatment process using soil mixed culture for Ammonia-N removal. Then, sample will be diluted for COD testing. The optimum conditions for the production of biogas from poultry manure wastewater will be determined using RSM. CCD will be applied to investigate the effects of two independent variables, namely agitation speed (rpm) and reaction time (days). Design Expert V7.0 will be used to design the experiment and analyse the experimental data. Spectrophotometer namely HACH DR5000 will be used to detect the COD of CM wastewater sample. The overall process performance will be measured by the yield of the biogas produced.

1.4 Main contribution of this work

The significant contribution of this research was to minimize the problem of inappropriate poultry manure wastewater management which cause negative impacts to the environment by foul odour transmissions, attraction of infectious microorganisms and runoff of nutrients into water sources (Kalyuzhnyi *et al.,* 1998; Atuanya and Aigbirior, 2002). Besides, biogas produced from AD is utilised worldwide to supply secure and economical energy.

In households, utilization of methane-rich biogas is initially contributed to cooking and lighting purpose in developing countries. The production of this renewable energy source is of household-scale common digesters with 2-10 m^3 volume which can only accommodate household energy consumption (Surendra *et al.,* 2014).

In industry, large-scale institutional biogas plants produce and purify biogas into bio-methane for other end-use purposes. The burning of biogas in combined heat and power (CHP) plants can generate electricity power supply for industrial and commercial areas. The waste heat also can be used for heating, drying or in refrigeration machines.

Moreover, biogas can be used as fuel for natural gas vehicles by the decoupling of production and utilisation (Lagerkvist *et al.,* 2012). The leftover digestate waste generated from AD can be used as bio-fertilizer for crops and plants in agricultural areas to improve the soil fertility (Zhang *et al*., 2007).

1.5 Organisation of this thesis

The organization structure of the thesis after introductory part was outlined as follow:

Chapter 2 provided the importance and application together with its background description on biogas and its applications in steam production, electricity generation, vehicle fuel and chemical production. Furthermore, this chapter delineated the potential of poultry manure wastewater in biogas production. Next, few potential feedstock for biogas production were reviewed. Besides, the AD technique used for biogas production from poultry manure wastewater includes hydrolysis, acidogenesis, acetogenesis and methanogenesis were briefly explained. Moreover, the modeling technique, RSM, used for optimization process with the aid of CCD in Design Expert Version 7.0 was described in detail. The mathematical analysis, ANOVA which aims to check the adequacy of model proposed also being discussed in the chapter. The factors affecting biogas production, which including agitation speed, reaction time, substrate-to-inoculum ratio, process system and type of substrate, were discussed in details with justification. This chapter ended with the selection of factors for optimization in this study.

Chapter 3 showed the methodology of the research work. It included the characterization and pre-treatment method of substrates. Besides, this chapter reviewed on the processes flow in AD experiment executed for biogas production from poultry manure wastewater by using RSM experimental design. In addition, COD-vial method analysis was described in details in this chapter. Next, validation run process also was being included in this chapter.

Chapter 4 devoted to the results data obtained from the experiment with the discussion as well as the comparison with other researchers. Besides, the characterization and pre-treatment results were discussed in the beginning of this chapter. The adequacy of proposed model was proved by mathematical analysis.

Chapter 5 summarized the research works covered in the body of this thesis includes a comprehensive summary of the findings. The thesis ended with recommendations which forecasting future works which might be developed in this work.

2 LITERATURE REVIEW

2.1 *Introduction to Biogas*

Biogas is a combustible mix of gases produced by AD of various forms of organic matter such as energy crops biomass (i.e., sugarcane and cassava) and waste materials (i.e., manure and sewage). Biogas is mainly composed of methane (CH_4) and carbon dioxide (CO_2) (Cvetković *et al.*, 2014). The characteristics of biogas such as it odor, exposure limits and impact on environment are shown in **Table 2.1**.

Table 2.1: Biogas characteristics (Grant and Marshalleck, 2008)

Biogas	Odor	Exposure Limit (ppm)	Environmental Impact
Methane (CH_4)	None	1000	GHG; explosive at 15 % mixture with air
Carbon dioxide (CO_2)	None	5000	GHG
Ammonia (NH_4)	Pungent	10	Acid rain when oxidized
Hydrogen sulphide (H_2S)	Rotten eggs	10	Highly flammable; acid rain when oxidized to sulfur

2.1.1 *Importance of biogas*

Biogas, a potential renewable energy source converts from biomass by AD, which acts a vital role to regulate the crisis of energy deficit and negative environmental effects (Schröder *et al.,* 2008; Rao *et al.,* 2010). The usage of biogas as a renewable energy source has great potential to minimize the emission of methane gas into environment (Cvetković *et al.*, 2014). Biogas has great potential to reduce global climate change. Since, the greenhouse effect for methane is 23 times higher than that of carbon dioxide (Gerlach *et al.,* 2013). The recovery of this significant energy by anaerobic treatment helps to reduce fossil fuel and greenhouse gases (GHG) (Gupta *et al.,* 2012). Biogas production has been paid close attention because of its potential as renewable and versatile energy source for heat and electricity generation, and transportation fuel (Lagerkvist *et al.*, 2012).

2.1.2 Application of Biogas

Biogas can be utilized for four main areas *viz.*, heat and steam production, electricity generation, vehicle fuel, and ultimately as feedstock for chemicals production. Primarily, the utilization of biogas in some developing countries only limited to cooking and lighting purpose because of moderate biogas digester size (Surendra *et al.*, 2014). Biogas provides higher energy content in cooking than fire fueled by traditional solid fuel resources. Lighting ranked second common usage of biogas right after cooking. In some regions out of electrical grid connection, biogas is introduced using special gas mantle lamps for lighting purpose (Singh and Sooch, 2004).

Besides, injection of upgraded biogas, named as biomethane, which meets stringent quality standards into the electrical grid helps to prevent contamination of the grid. The only biogas component that contributes as energy carrier is CH_4 (Surendra *et al.*, 2014). The energy content for pure biomethane is approximate to be 10 kWh/m^3 while that for biogas with assumption of 60 % methane content is 6 kWh/Nm^3 (Appels *et al*., 2008).

Biogas acts as an ideal fuel in CHP applications all around the world. The mechanism of these combine engines is generated by the conversion of mechanical power into electricity. Biogas can replace fossil fuels to drive natural gas vehicles after it is upgraded to have same quality as natural gas. For example, in Pura, India, a biogas project was implemented by the community in which a modified diesel engine and an electrical generator were successfully powered by a biogas digester (Reddy, 2004).

2.2 Feedstock for biogas production

For biogas production from organic matter, its appropriate raw material must be suitable for AD process. There are a variety of feedstock such as animal manure, municipal wastewater and agricultural crops residues. The sustainability of biogas production must consider on a few aspects include energy potential, environment, and economic feasibility (Cvetković *et al.*, 2014). In the following section, the review of various feedstock was discussed.

2.2.1 Poultry Manure Wastewater

Poultry manure wastewater is one of the most abundant biodegradable materials accumulating in local poultry farming area and has good potential to produce biogas. Generally, poultry manure includes faeces and urine excreted by chicken, which contains high organic nutrients and has high fertilizer value on crops in agricultural areas. The CM production in one poultry farm at California is illustrated in Figure 2.1

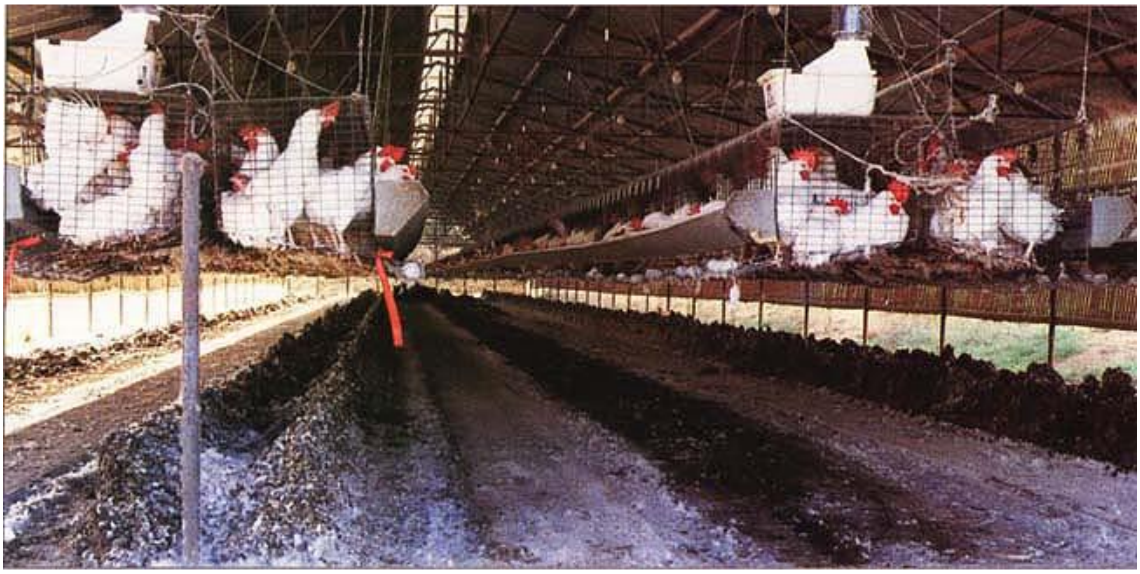

Figure 2.1: CM production on California poultry farm (Mullens et al., 2001)

A good management of this waste can augment high cost commercial fertilizers (Moreki and Chiripasi, 2011). The tremendous expansion of poultry population is due to the increasing demand for chicken products. As a side effect, the amount of poultry excrement is also rising. An inappropriate disposal and treatment of these poultry manure can cause spread of diseases, soil and groundwater pollution and risks the health and environment (Roeper *et al.,* 2005). Poultry manure is mainly categorized in solid, slurry and wastewater. Collection of CM is commonly the mixture of solid form and other chicken production residues (Haga, 2001).

Poultry manure is richer in biodegradable organic nutrient than other animal wastes (Hill, 1983; Morris *et al*., 1975). The AD of fresh poultry manure will decrease the process efficiency due to ammonia accumulation in high solid content, therefore the treatment of poultry manure in its semi-solid state has been experimented (Bujoczek *et al*., 2000).

2.2.2 Municipal Solid Waste

Municipal solid waste (MSW) is waste collected mainly from households, non-hazardous solid waste from commerce and trade, offices and institutional establishment including hospitals, wastes from market and yard, and even sweepings from streets (Ogwueleka, 2009). Composition and quantity of MSW can be determined from the living habits and standard of community. The quantification unit used for MSW is expressed in kg/person/year, which indicating the waste generated per person in a year (Cvetković *et al.*, 2014).

Kiely (1997) defined solid wastes to include activities from human and animal and also liquid wastes such as paints, old medicines and spent oils. This shows the possibility of intermixing between both solid and liquid wastes. However, the study found the MSW as largely static which emerged as one of the greatest challenges in its handling and management. A proper disposal management can prevent many environmental problems such as unpleasant odours and blockage of water drain ways which might further lead to pollution and flooding respectively (Igoni *et el.*, 2008). The example of one disposal area of MSW in Malaysia is shown in **Figure 2.2**.

Figure 2.2: Municipal solid waste in Malaysia in 2013 (Eckard, 2013)

Conventionally, MSW disposal has been mainly managed by land filling. However, the anthropogenic methane emission from the landfills waste has been identified as essential contributor to global warming (Stocker *et al.*, 2013). AD of MSW has been emphasized as one of the acceptable treatment to reduce and stabilize solid waste volume for biogas production (Stroot *et al.*, 2001).

2.2.3 Agricultural wastes

Agricultural waste describes both organic and non-organic wastes produce on an agriculture farm through various farming activities. Horticulture, dairy farming, seed growing, grazing land, livestock breeding, nursery plots and woodlands are among the examples of agricultural activities (Ashworth and Azevedo, 2009). Agricultural wastes such as crop residues, wood and other plant residues are highly energy rich and inexpensive for fermentation. Some of the famous substrate used for AD to produce biogas includes sugarcane bagasse, rice straw, cassava waste, palm oil mill waste, and wheat bran (Ezejiofor et al., 2014). The sugarcane bagasse is shown in **Figure 2.3**. The agricultural wastes production in Indonesia in year 1989 has shown in **Table 2.2**.

Figure 2.3: Sugarcane bagasse (Openpr.com, 2008)

Table 2.2: Production of agricultural wastes in year 1989 (Dewi and Siagian, 1992)

Type of Agricultural wastes	**Production (ton/year)**
Rice straw	44,723,000
Sugarcane bagasse	8,561,606
Cassava waste (root shell and stalk)	6,713,000

The potentiality of agricultural wastes for biogas production can reduce environmental pollution and also minimize the utilization of commercial energy source such as kerosene and firewood. These can be proved via some examples of the process applications for biogas production. Firstly is the cassava waste treatment to reduce polluted river near tapioca starch industry. Secondly, the utilization of water hyacinth as substrate for AD can solve Curug dam problem in Indonesia (Ishizuka *et al.*, 2010).

The biogas production by AD of agricultural wastes is done via the synergistic action of a consortium of hydrogenic, acidogenic, acetogenic and methanogenic bacteria (Amigun *et al.*, 2008). Although agricultural wastes are one of the potential feedstock for AD to produce biogas, but it still possesses some limitations. The main problem with AD of agricultural wastes is that it contains high cellulose levels, hemicellulose, starch, lipids and proteins (Oliveira and Franca, 2009). This speciality and complexity structure makes cellulose resistant to both biological and chemical treatments (Taherzadeh and Karimi, 2008). The lignocelluloses degradation makes the hydrolysis stage slower and rate limiting. Therefore, agricultural wastes substrate needs to practice chemically or mechanically pre-treatment to ease the accessibility for microbial growth in AD process (Ezejiofor et al., 2014).

2.3 Technique used for Biogas Production

AD is one of the advantageous and beneficial processes used for biogas production from poultry manure (Sakar *et al.*, 2009). Generally, the biogas production from AD using the concept of biomethanation of animal manure yield principal gases *viz.*, methane and carbon dioxide (Rao *et al.*, 2010). The biogas yield will depends on substrate mix and several operating conditions such as incubation time and temperature (Olsson and Fallde, 2014). Biogas production can reduce nuisance odors in agricultural farms (Schröder *et al.*, 2008). Beside the function of stabilization and deodorization of poultry manure, AD also turns poultry manure, which initially acts as natural fertilizers into easy-disposable organic fertilizers (Borowski *et al*., 2014). Biogas produced from AD can be utilized as cooking gas and fuel, the digestate become bio-fertilizer and the sludge component can used as a soil conditioner after dried (Zhang *et al*., 2007). AD is a natural established bioconversion technology which follows a sequence of reactions which are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Poh and Chong, 2009). These interdependent reactions are occurs simultaneously and synergistically as shown in **Figure 2.4**.

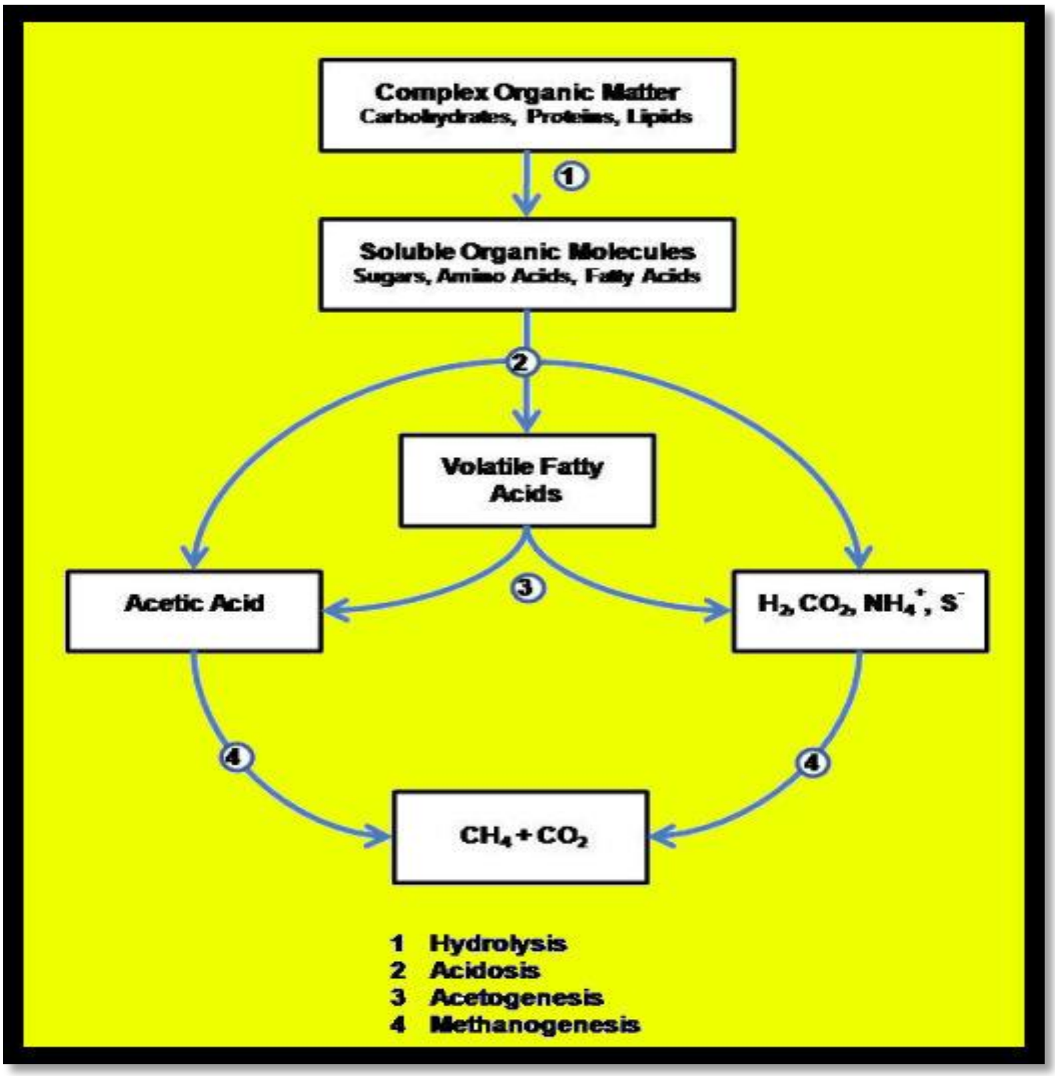

Figure 2.4: Step in AD (Hamilton and Ciolcosz, 2010).

2.3.1 Hydrolysis

In AD, hydrolysis breaks down insoluble and complex organic molecules such as lipids, carbohydrates and proteins into water soluble, simpler organic molecules such as sugars and amino acids by enzyme (Hamilton and Ciolcosz, 2010). Proteins provide a source of carbon, nitrogen and energy for the growth of bacteria in anaerobic digesters (Palmisano & Barlaz, 1996).

Hydrolysis is considered as the time limiting step for solid matter degradation. This is because some solid matter such as lignin and cellulose might be non-degradable during AD. Consequently, woody waste such as straw residues which is rich in lignin content is not an ideal feedstock to undergo AD process as its phenolic groups might be inhibitory to the enzymes (Arsova, 2010). The other way round, acid development will be fastening up if major content of easily degradable carbohydrates like glycogen or starch in the substrate.

In this stage, the enzymes involved such as lipases, cellulase and protease are produced by fermentative and hydrolytic bacteria (Arsova, 2010). These bacteria play important role to depolymerize organic matter towards their monomer compounds. Basically, extracellular lipases and phospholipases take the role to hydrolyze fats into glycerine, alcohols and fatty acids (Dornack, 2012). Besides, proteolytic enzymes produced by anaerobic bacteria are responsible to hydrolyze proteins to peptides, amino acids and carbon dioxide (Palmisano & Barlaz, 1996).

An example of hydrolysis break down of organic waste into simple sugar, in this case glucose is shown in equation (2.1) in which n indicates the stoichiometric coefficient of respective compounds. On the other hand, degradation of proteins into their constituent amino acid and lipids into long chain fatty acids are occur under similar reactions.

$$[C_6H_{10}O_4]n + 2nH_2O \rightarrow n[C_6H_{12}O_6] + nH_2 \qquad (2.1)$$

2.3.2 Acidogenesis

Acidogenesis then convert soluble organic molecules into volatile fatty acids. The anaerobic fermentation happens by the combination of hydrolysis and acidogenesis (Hamilton and Ciolcosz, 2010). In this stage, the acid formers microorganisms transform hydrolytic products such as sugars, amino acids, alcohols and fatty acids into simple organic acids. The fermentation products varying by the concentration of intermediary bound hydrogen. A high pH values lead to reduced end products such as propionic acid and butyric acid whereas a low pH values lead to an intensified production of acetate, carbon dioxide and hydrogen (Palmisano & Barlaz, 1996).

Equation (2.2) and (2.3) shows the examples of typical acidogenesis reactions, where glucose is transformed into ethanol and propionic acid, respectively (Ostrem, 2004).

Conversion of glucose to ethanol

$$C_6H_{12}O_6 \leftrightarrow 2\ CH_3CH_2OH + 2CO_2 \qquad (2.2)$$

Conversion of glucose to propionic acid

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2\ H_2O \quad (2.3)$$

2.3.3 *Acetogenesis*

After that, in acetogenesis, the intermediate volatile fatty acids are further converted into acetate and a gas composition of hydrogen, carbon dioxide, ammonia and hydrogen sulfide by acetogenic bacteria (Hamilton and Ciolcosz, 2010). Basically, this pathway of single acid forming stage aims to reduce biochemical oxygen demand (BOD) and COD values. Generally, high pH values inhibit the growth rate of acetogenic bacteria. Acetogenic bacteria are also known as obligatory hydrogen-producing acetogens (OHPA) as they exhibit a metabolism of proton reduction and are mandatory dependent on hydrogen removal (Arsova, 2010). Therefore, there is a close special symbiosis between acetogenesis and methanogenesis to ensure the direct utilization of the hydrogen developed.

Practically, some fermentation products such as alcohols, propionic acid and butyric acid formed within this stage as a mechanism to remove accumulating electrons and hydrogen. In this stage, some of the important reactions for the formation of acetic acid are as follow, which are mainly from the conversion of glucose (equation 2.4), ethanol (equation 2.5) and propionic acid (equation 2.6).

Conversion of glucose to acetic acid

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.4)$$

Conversion of ethanol to acetic acid

$$CH_3CH_2OH + H_2O \leftrightarrow CH_3COOH + 2H_2 \quad (2.5)$$

Conversion of propionic acid to acetic acid

$$CH_3CH_2COOH + 2H_2O \leftrightarrow CH_3COOH + CO_2 + 3H_2 \quad (2.6)$$

Hydrogen plays a crucial intermediary role in acetogenesis as all the acids conversion happen under low hydrogen partial pressure condition. The thermodynamic feasibility of acetogenesis is sustained by the presence of hydrogen scavenging bacteria (hydrogenotrophs) which lowering the partial pressure of hydrogen (Ostrem, 2004).

Hydrogenotrophs are pH sensitive bacteria. Whenever the conditions within the anaerobic digester result in a pH drop, hydrogen will be stored within propionic acid by ecology response. This reversal of the reaction to achieve equilibrium can be explained in Le Chatelier's principle.

The health condition of an anaerobic digester is indicated by its low hydrogen concentration. This is also the reason in which hydrogen only appears as a trace component in biogas. Acetogenesis along with acidogenesis represents the transition from soluble organic molecules to the methanogenic substrate.

2.3.4 Methanogenesis

In last stage which is methanogenesis, then substrate for methanogenic microorganisms release biogas, which include methane and carbon dioxide as principal products (Hamilton and Ciolcosz, 2010). Methane, the main component in biogas, is produced through a syntrophic relationship between acetate-oxidizing bacteria and hydrogen-utilizing methanogens (Arsova, 2010). Acetotrophic or acetoclastic methanogens convert acetic acids to methane and carbon dioxide via decarboxylation of acetic acid as shown in equation (2.7). The second type of anaerobic archaea is called hydrogenotrophic or hydrolytic methanogens reduce carbon dioxide and hydrogen into methane and water using H_2 as electron donor as shown in equation (2.8) (O'Flaherty *et al.*, 2006; Hamilton and Ciolcosz, 2010).

Acetic acid cleavage

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (2.7)$$

Carbon dioxide reduction

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (2.8)$$

Ordinarily, methanogenesis process occurs naturally in manures, agricultural fields and aquatic sediments, and plays a vital role for the carbon cycle to sustain the ecosystem (Arsova, 2010). Stabilization is said to be achieved when methane and carbon dioxide are produced. The archeabacter genus methanogenic bacteria are mainly categorized according to their shape. According to Eggeling *et al.* (1986), Methanosarcina genus is in spherically shaped, Methanothrix bacteria is in long and tubular shaped and bacteria that catabolize furfural and sulfates appeared as short and curved rods as illustrated in **Figure 2.5**.

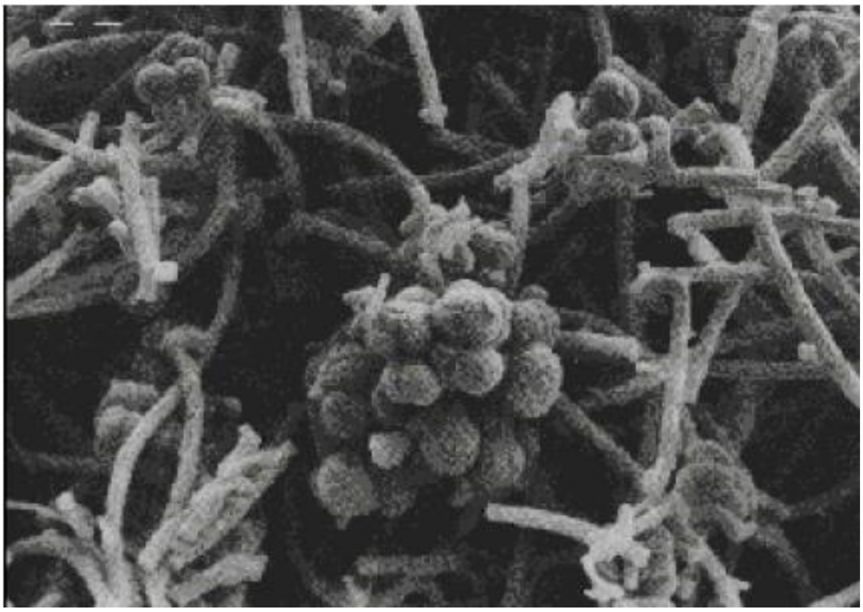

Figure 2.5: Common methanogenic bacteria in methane formation process (Eggeling et al., 1986).

2.4 Optimization Using RSM

RSM is a widely used modelling technique functioned to develop, improve and optimize the response variable in the statistical design of experiments (Ba ş and Boyacı, 2007). RSM is applicable when a response of interest is influenced by several parameters or variables and the objective is to optimize this response. It can be expressed as

$$y = f(x_1, x_2) + e \qquad (2.9)$$

where the response y depends on independent variables x_1 and x_2, and the experimental error denoted as e.

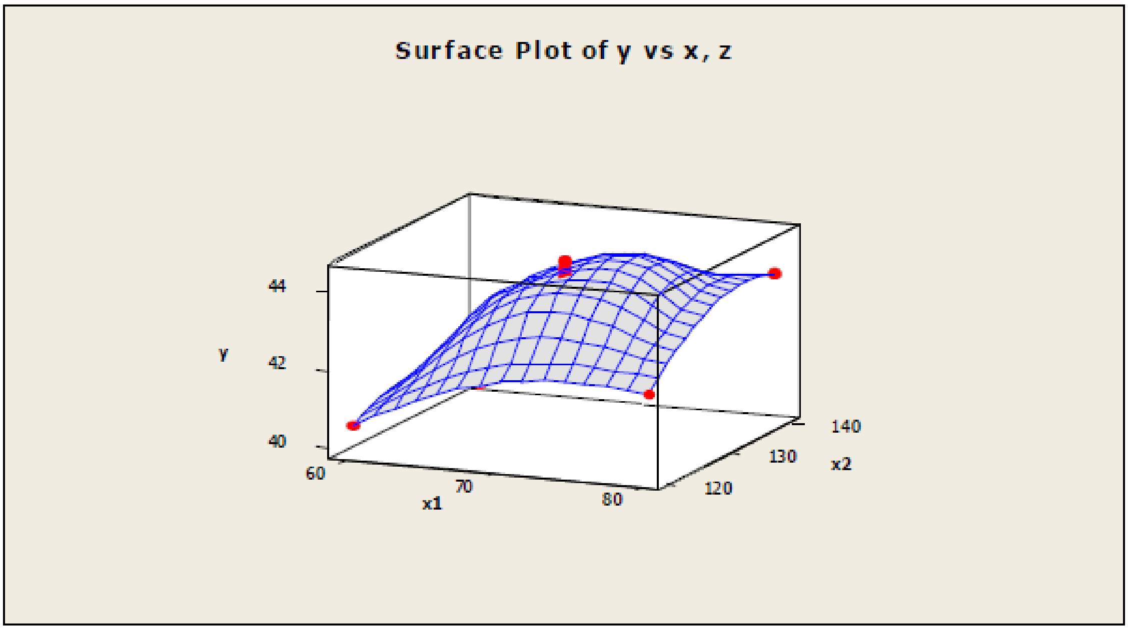

Figure 2.6: Response surface plot.

From the example of three-dimensional response surface plot in **Figure 2.6**, the optimal response can be visualized its respective value on independent variables (Bradley, 2007). The proper analysis of RSM will shows the local maximum, local minimum and ridge lines on the topography of response surface and identifies the optimal response region for the design (Olayiwola *et al*., 2011; Montgomery, 2001).

Design Expert Version 7.0 is software which applies important statistical and mathematical methods to find the best model to describe the response data. A three dimensional surface graph for the responses will be modelled out where the optimization point can be easily obtained from (Ba ş and Boyacı, 2007). There are several types of design of RSM such as three-level factorial, Box-Behnken, D-Optimal and CCD.

2.4.1 Central Composite Design

According to Bezerra *et al.* (2008), CCD is the most employed design of optimization for the development of analytical procedures compared to the others as their low efficiency of the latter especially for a numbers of variables. CCD is a second order factorial design utilized in RSM since full factorial design (FFD) possessed too large number of runs which is less practical (Box and Wilson, 1951).